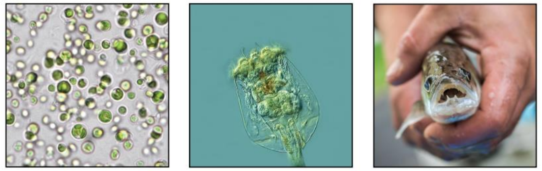
EVROPSKÁ UNIE
Interreg
EVROPSKÁ UNIE
Rakousko-Česká republika
Evropský fond pro regionální rozvoj

Selection of microalgae as a feed for rotifers and pikeperch larvae



KAROLÍNA ŠTĚRBOVÁ

IMIC, CENTRE ALGATECH, TŘEBOŇ

ALGATECH
IMIC
BEST
Bioenergy and
Sustainable Technologies
Bundesamt
für Wasserwirtschaft
Fakulta rybářství
a ochrany vod
Faculty of Fisheries
and Protection
of Waters
Jihočeská univerzita
v Českých Budějovicích
University of South Bohemia
in České Budějovice

MAIN GOALS OF THE PROJECT

JOINT RESEARCH

Fakulta rybářství
a ochrany vod
Faculty of Fisheries
and Protection
of Waters

Jihočeská univerzita
v Českých Budějovicích
University of South Bohemia
in České Budějovice

IMIC

ALGATECH

BEST
Bioenergy and
Sustainable Technologies

Federal Agency
for Water Management

DEMONSTRATE THE ECO-INOVATIVE TECHNOLOGY USING WASTE NUTRIENTS

Further tasks

- Selection of **microalgae** strains with a **specific fatty acid profile** (C18:2n6, LA; C18:3n6, GLA; C18:3n6, ALA)
- **Cultivation** of selected microalgae **in eco-inovative unit** (characterization, biomass analysis)
- **Providing** the microalgae biomass **to FF Vodňany** for further experiments

THEORETICAL BACKGROUND

Fatty acids synthesis pathway

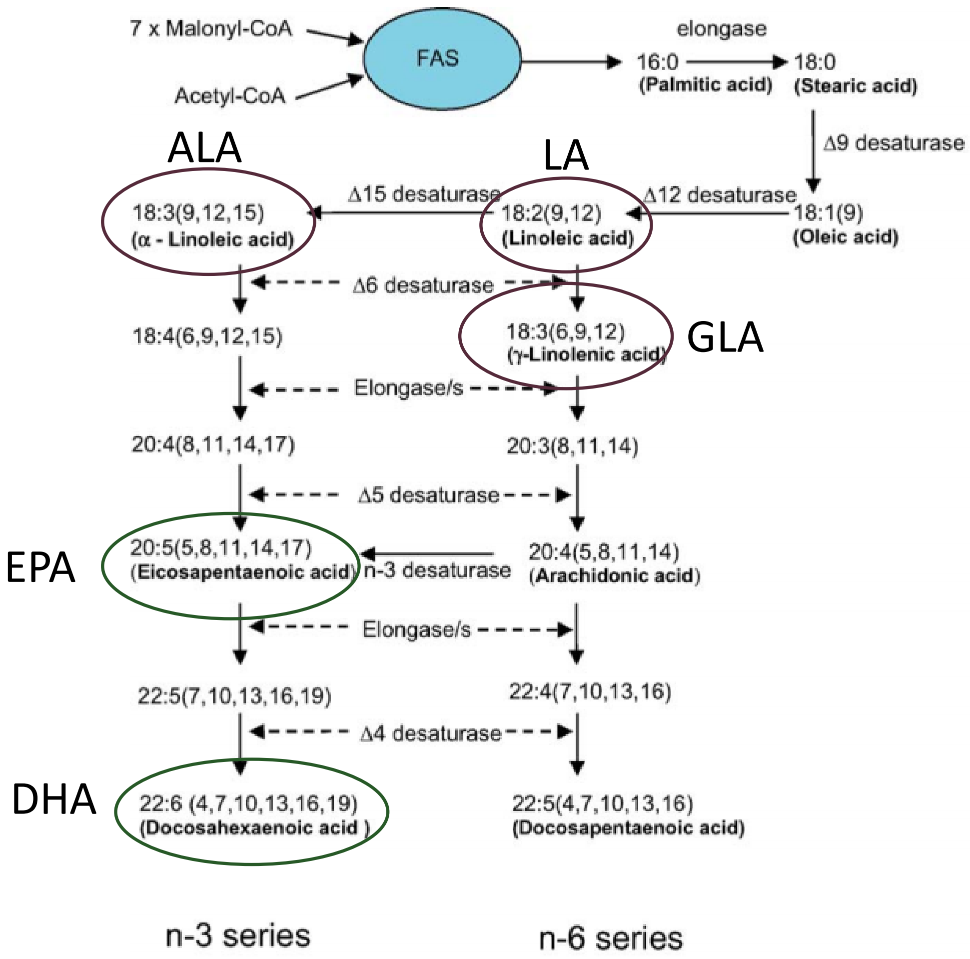


Fig. 1 Synthesis pf fatty acids.

515 USD/1kg DW

*https://reedmariculture.com/products/nanno

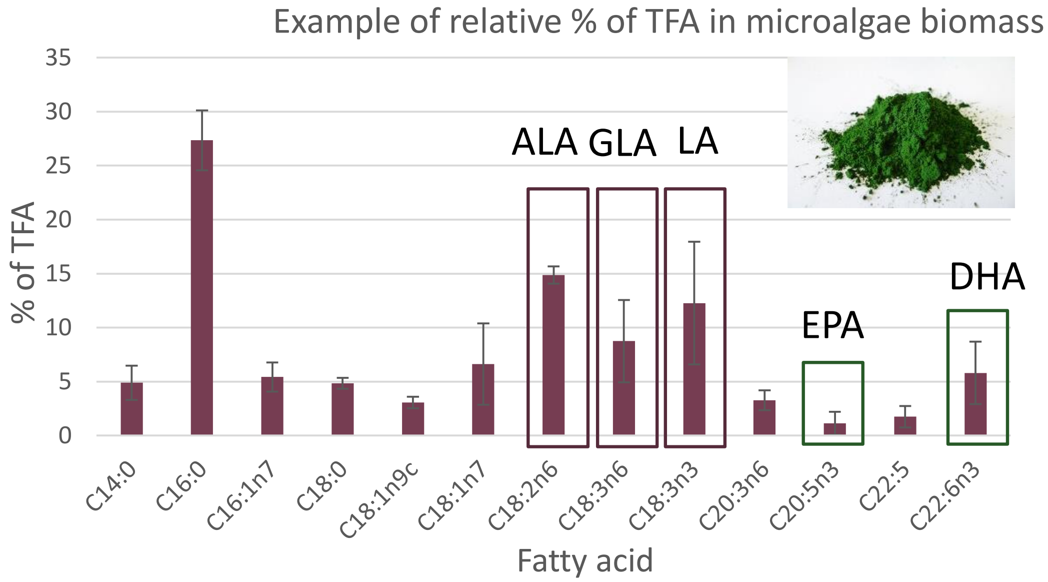


Fig. 2 General fatty acids analysed by GC-FID.

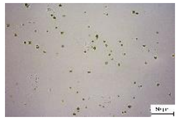
SELECTION OF MICROALGAE

- ○ Literature
- ○ Knowledge
- ○ Recommendation
- ○ Preliminary experiments

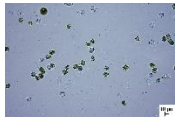
Fig. 3 Preliminary selection of *Monodopsis* strain from total 9 strain based on the growth rate.

CHLOROPHYCEAE

- ○ *Chlorella vulgaris*



- ○ *Monodopsis* SVB200



- ○ *Monoraphidium* „B“

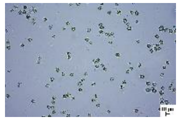
LA

ALA

GLA

EUSTIGMATOPHYCEAE

- ○ *Trachydiscus minutus*



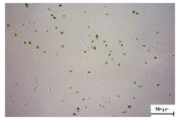
- ○ *Vischeria helvetica*

+EPA

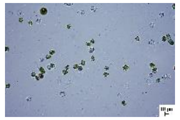
PROVIDING OF MICROALGAE TO BEST

CHLOROPHYCEAE

- ○ *Chlorella vulgaris*



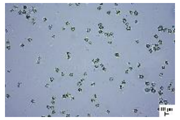
- ○ *Monodopsis* SVB200



- ○ *Monoraphidium* „B“

EUSTIGMATOPHYCEAE

- ○ *Trachydiscus minutus*



- ○ *Vischeria helvetica*

ALL STRAINS PROVIDED TO BEST FOR FURTHER EXPERIMENTS IN DIGESTATE

SELECTION OF BEST CULTIVATION MEDIA

Growth tests in a small scale

BBM vs. 6N BBM vs. medium with urea vs. BG-11

Fig. 4 Selection of best growing media for selected microalgae strains in 100mL cylinders.

Tab. 1 Growth rate [d^{-1}] of selected microalgae strains in different cultivation media.

Microalgae strain/medium	Growth rate [d^{-1}]			
	BBM	**6N BBM**	**UREA**	**BG-11**
Chlorella vulgaris	0.41±0.05	0.37±0.05	0.43±0.05	0.43±0.05
Monodopsis	0.32±0.00	0.25±0.01	0.14±0.01	0.40±0.01
Monoraphidium „B“	0.27±0.03	0.33±0.03	0.22±0.05	0.36±0.04
Trachydiscus minutus	0.35±0.02	0.32±0.02	0.39±0.04	0.42±0.04
Vischeria helvetica	0.31±0.01	0.27±0.02	0.29±0.02	0.29±0.02

CULTIVATION IN MUNICIPAL WASTEWATER

SMALL CYLINDERS

BG-11 vs. municipal wastewater (WW)

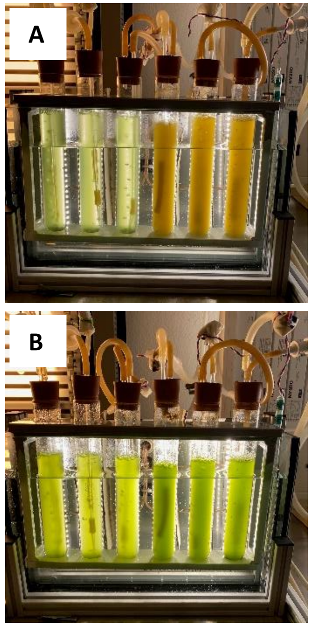


Fig. 5 Growing of *Chlorella vulgaris* in BG-11 medium and municipal wastewater A) t=0h, B) t=19h.

Tab. 2 Growth rate [d^{-1}] of selected microalgae strains in wastewater (WW) compared to the growth in BG-11 medium.

[mg L^{-1}]	N-NO$_3$	N-NH$_4$	TN	P-PO$_4$	TP
BG-11	250	-	**250**	7	**7**
WW	0.11	**207**	**273**	163	**183**

Tab. 3 Growth rate [d^{-1}] of selected microalgae strains in wastewater (WW) compared to the growth in BG-11 medium.

Microalgae strain/medium	Growth rate [d^{-1}]	
	BG-11	**WW**
Chlorella vulgaris	0.32±0.0	0.25±0.05
Monodopsis	0.32±0.03	0.27±0.01
Monoraphidium „B“	0.28±0.03	0.19±0.08
Trachydiscus minutus	0.40±0.00	0.38±0.02
Vischeria helvetica	0.35±0.01	0.33±0.03

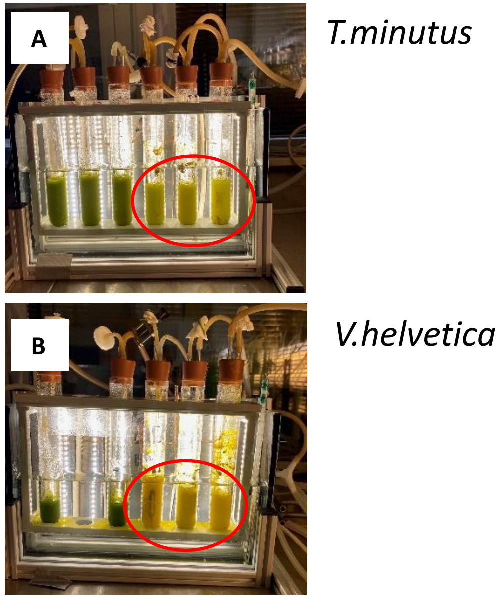


Fig. 6 Cultivation of A) *Trachydiscus minutus* and B) *Vischeria helvetica* in BG-11 (first three cylindres from left) and WW (right three cylinders).

CULTIVATION IN MUNICIPAL WASTEWATER

BIGGER 400 ML CYLINDERS

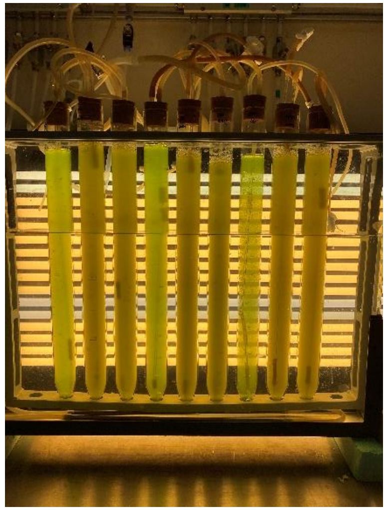


Fig. 7 Growing of *selected* microalgae in municipal wastewater in 400mL cylinders.

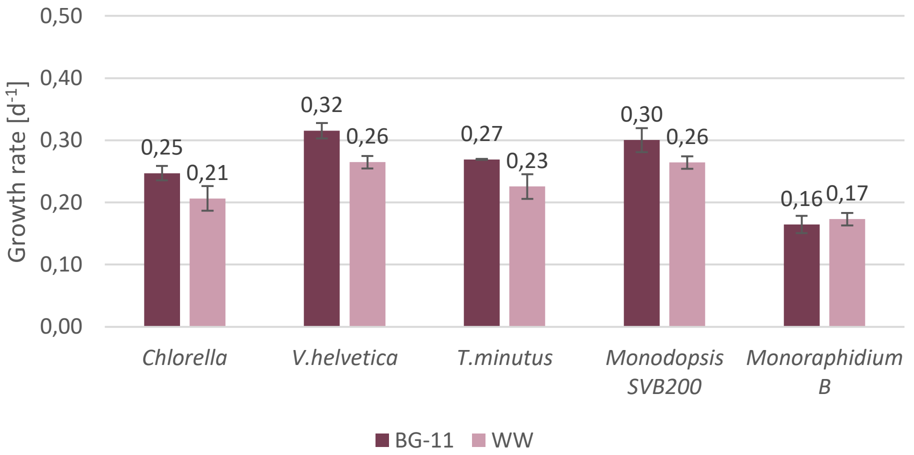


Fig. 8 Growth rate of selected microalgae in BG-11 medium and wastewater (WW).

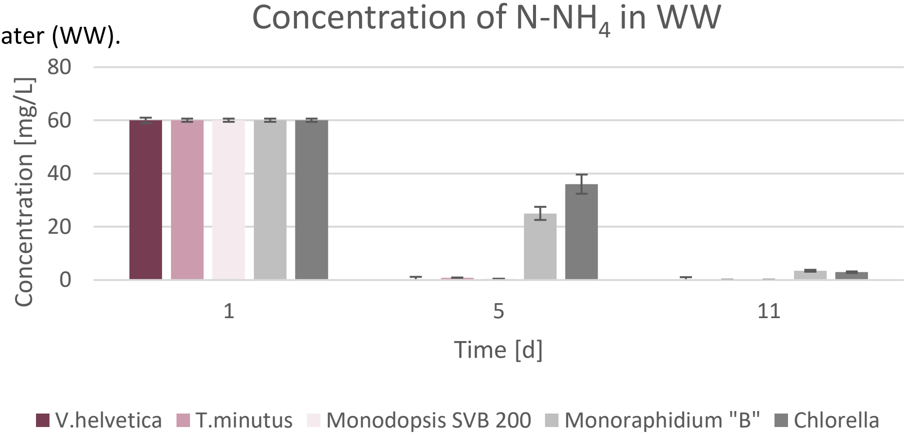


Fig. 9 Ammonium (NH_4^-) removal from wastewater (WW).

CULTIVATION IN DIGESTATE

from Třeboň‘s biogass station

Fig. 10 Digestate pre-treatment.

Tab. 4 Chemical analysis of digestate used for microalgae cultivation.

Sample/measured parameter	TS	BOD 5	COD Cr	TOC	N-NO$_3$	N-NO$_2$	N-NH$_4$	N-tot	P-PO$_4$	P - tot
	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
Digestate 50x diluted	15	110	1300	630	0.38	<0,002	45	120	4.3	9.8
Digestate	750	5500	65000	31500	19	0.1	2250	6000	215	490
BG-11	0	-	-	-	250	-	-	250	7	7

Fig. 11 Preliminary experiments with digestate in 400mL cylinders.

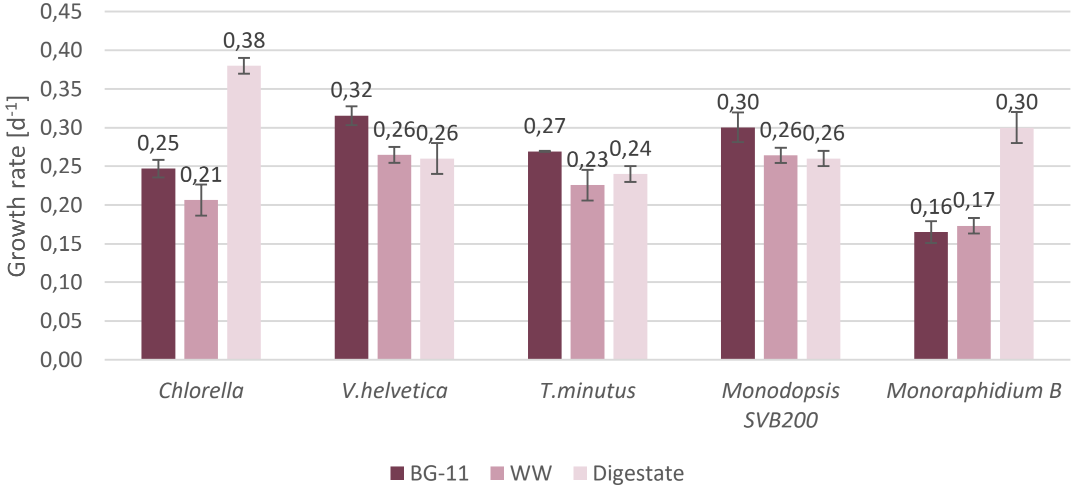


Fig. 12 Growth rate of selected microalgae in BG-11 medium, wastewater (WW) and digestate.

ASSEMBLING OF ECO-INOVATIVE UNIT (AC-PBR)

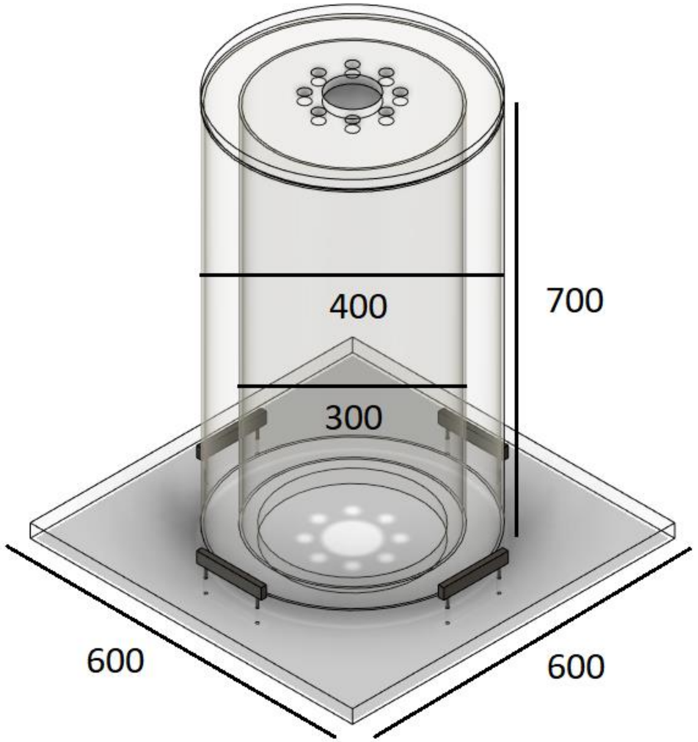


Fig. 13 Schema of annular-column photobioreactor (AC-PBR).

Fig. 14 Assembling of AC-PBR.

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a ochrany vod
Faculty of Fisheries
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Jihočeská univerzita
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University of South Bohemia
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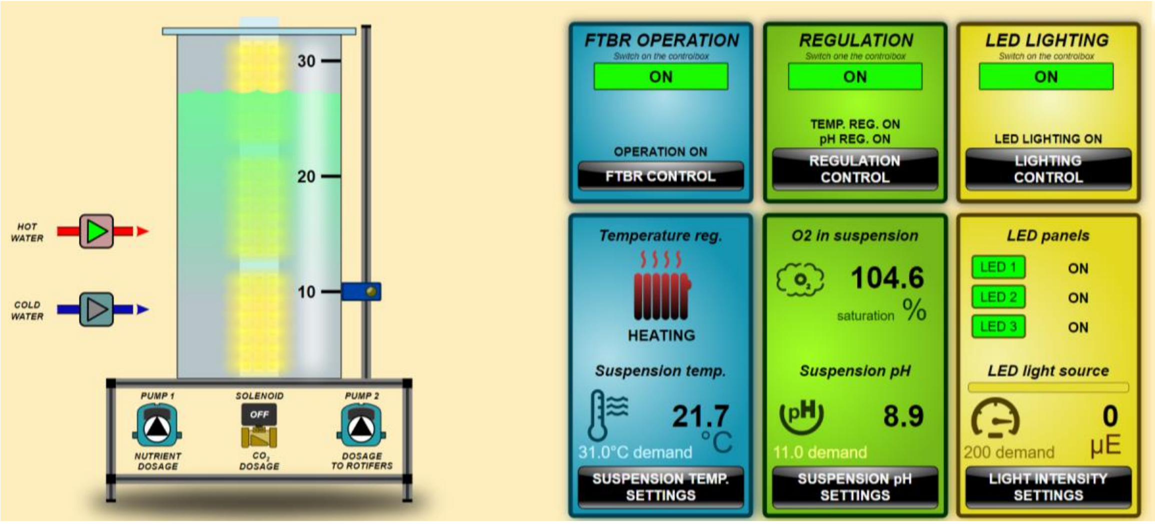
Bundesamt
für Wasserwirtschaft

ASSEMBLING OF ECO-INOVATIVE UNIT (AC-PBR)

Fig. 15 Testing of AC-PBR.

BASIC SPECIFICATION

- V = 30L
- Photic layer = 45mm
- Bubbling and cooling loop
- Automatic CO_2 addition (pH-stat)
- On-line monitoring of variables
- Maximum LI=1600 µmol m^{-2} s^{-1}
- pH, O2, t, pumps regulation
- Turbidity probe SOON



FTBR OPERATION
Switch on the controlbox
ON
OPERATION ON

REGULATION
Switch one the controlbox
ON
TEMP. REG. ON
pH REG. ON

LED LIGHTING
Switch on the controlbox
ON
LED LIGHTING ON

Temperature reg.
HEATING
Suspension temp.
21.7 °C
31.0°C demand

O2 in suspension
104.6
saturation %
Suspension pH
8.9
11.0 demand

LED panels
LED 1 ON
LED 2 ON
LED 3 ON
LED light source
0 µE
200 demand

Fig. 16 Control software of AC-PBR (accesible on http://147.231.249.16/)

HANDOVER OF AC-PBR

18.1.2022
Scharfling, Mondsee

Fig. 17 Handover of AC-PBR in Scharfling.

21.1.2022
Faculty of Fisheries, Vodňany

Fig. 18 Handover of AC-PBR in Vodňany.

CULTIVATION IN ECO-INOVATIVE UNIT

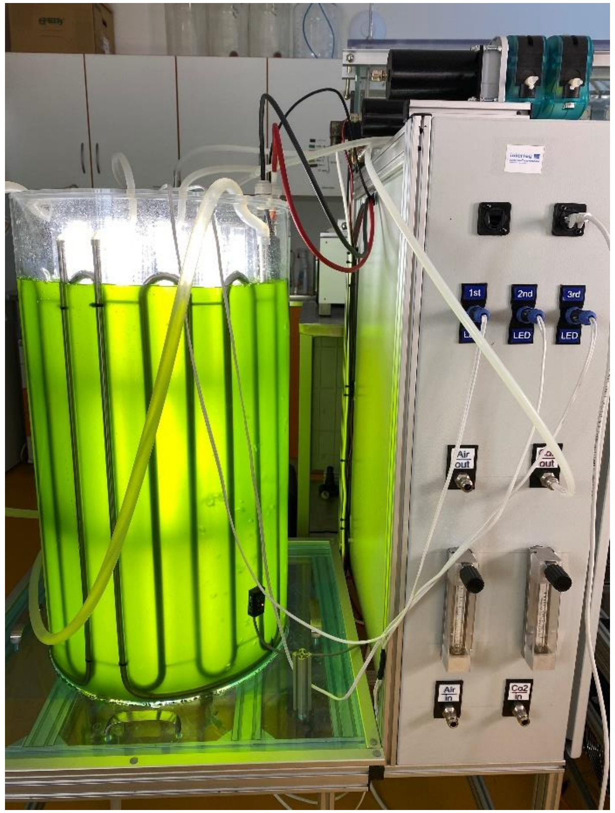


Fig. 19 First cultivation in AC-PBR.

CHLOROPHYCEAE

- *Chlorella vulgaris*
- *Monodopsis* SVB200
- *Monoraphidium* „B“

EUSTIGMATOPHYCEAE

- *Trachydiscus minutus*
- *Vischeria helvetica*

MEASURED VALUES

- Dry weight
- Cell numbers
- Photosynthesis performance
- Pigments (Chl *a*, Chl *b*, Car)
- Fatty acids

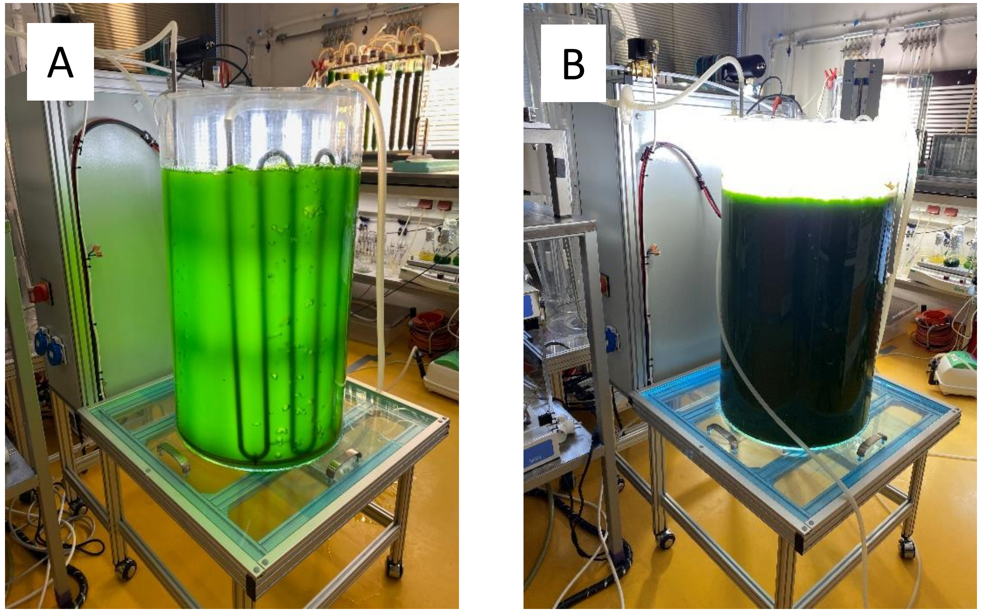


Fig. 20 Cultivation of *Chlorella vulgaris* in AC-PBR A) D1 and B) D15.

EXPERIMENTS IN AC-PBR

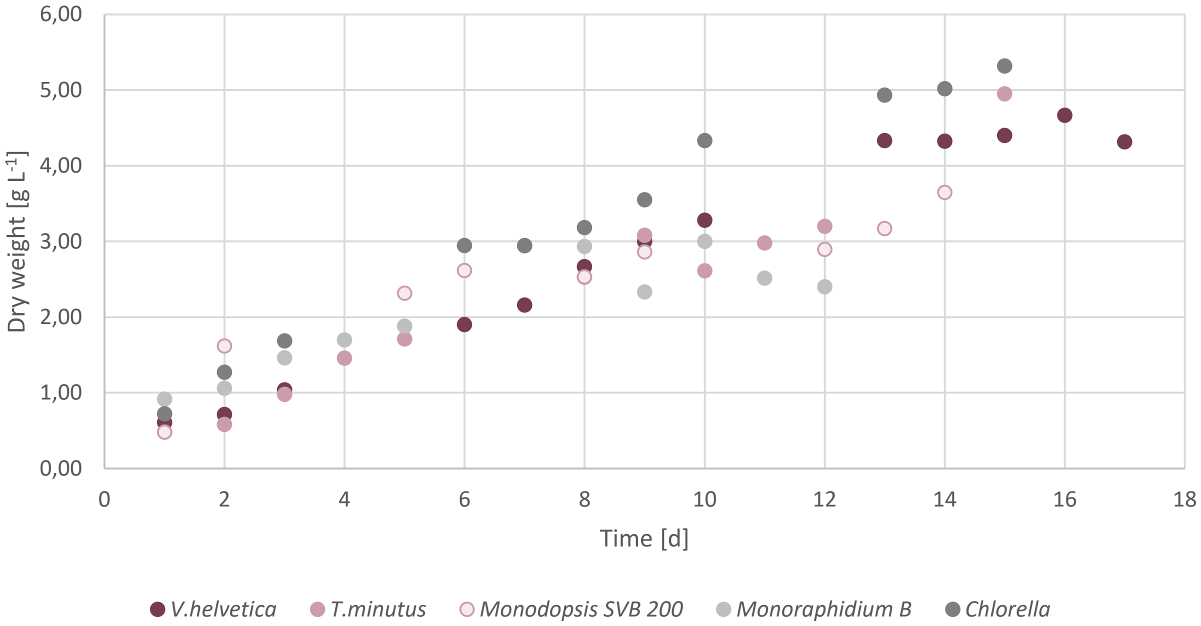


Fig. 21 Growth curves of microalgae cultivated in AC-PBR.

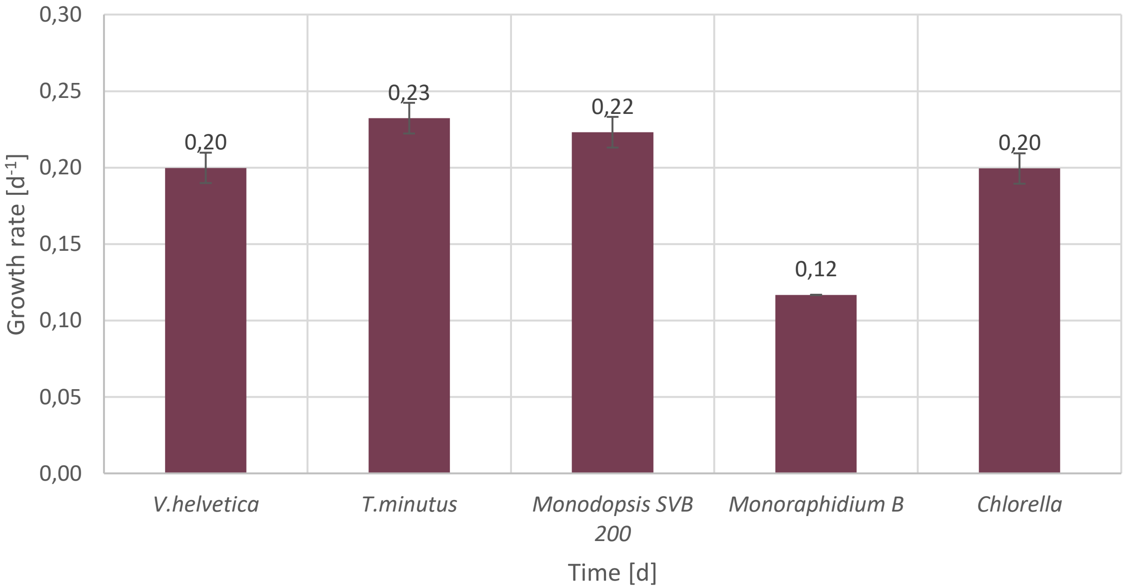


Fig. 22 Growth rates [d^{-1}] of microalgae cultivated in AC-PBR.

FLUORESCENCE MEASUREMENTS

Light Response Curve (LRC)

Kautsky Curve (OJIP)

Oxygen Evolution (POE)

- Relationship between LI and photosynthetic rate

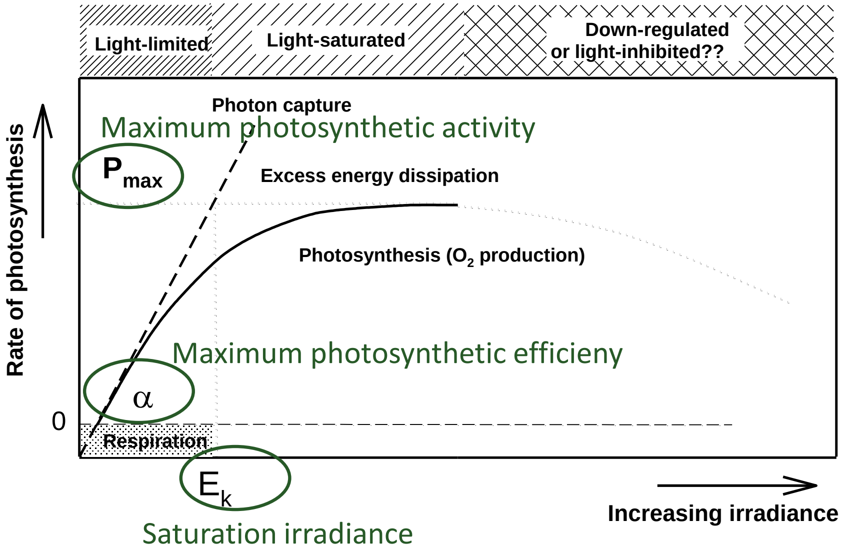


Fig. 23 Theoretical explanation of light repsonse curve (LRC).

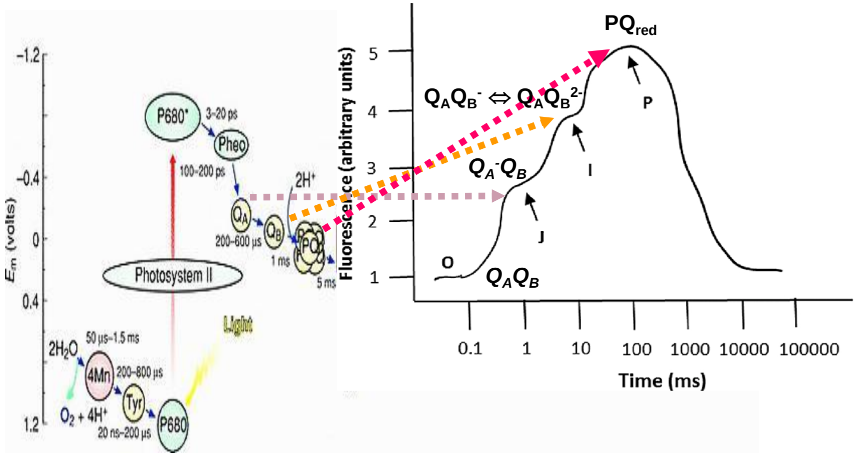


Fig. 24 Transport of electrons in PQ pool.

Vj, Vi

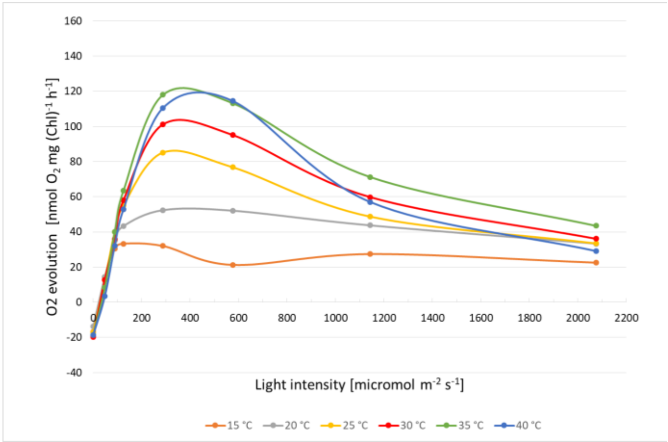
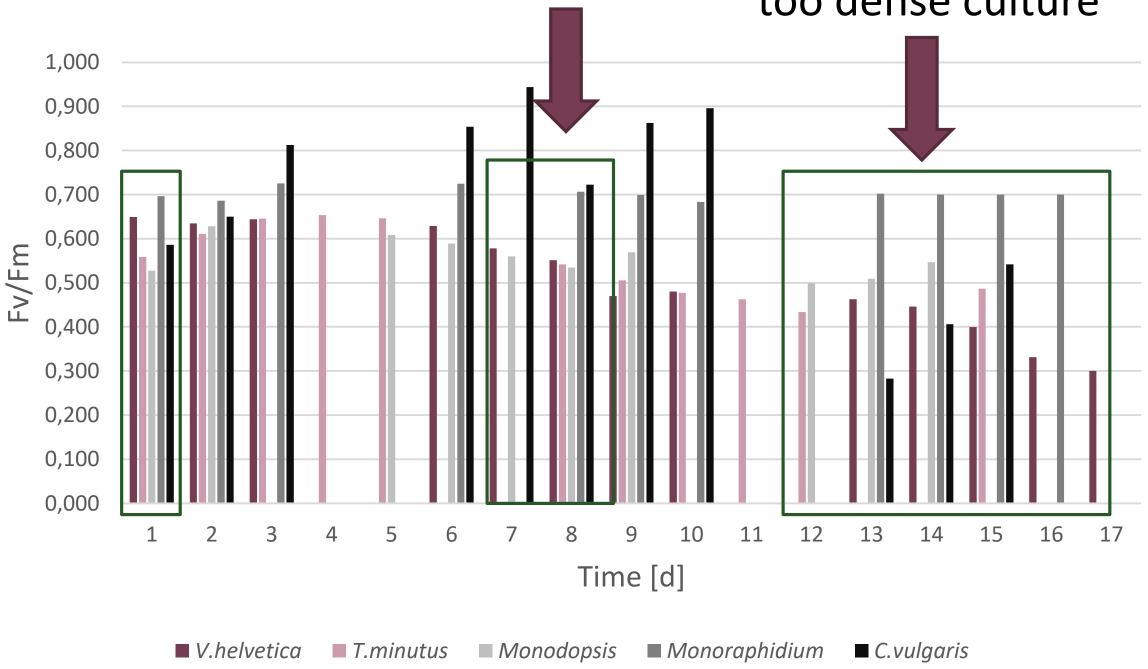


Fig. 25 Photosynthetic oxygen evolution.

FLUORESCENCE MEASUREMENTS



LI increase

too dense culture

Fig. 26 Maximum photochemical yield of selected microalgae grown in AC-PBR.

Fv/Fm = 0.7-0.8

healthy, non-stressed culture

Maximum photochemical yield

According Fv/Fm response we can adjust the LI

FATTY ACID PROFILE

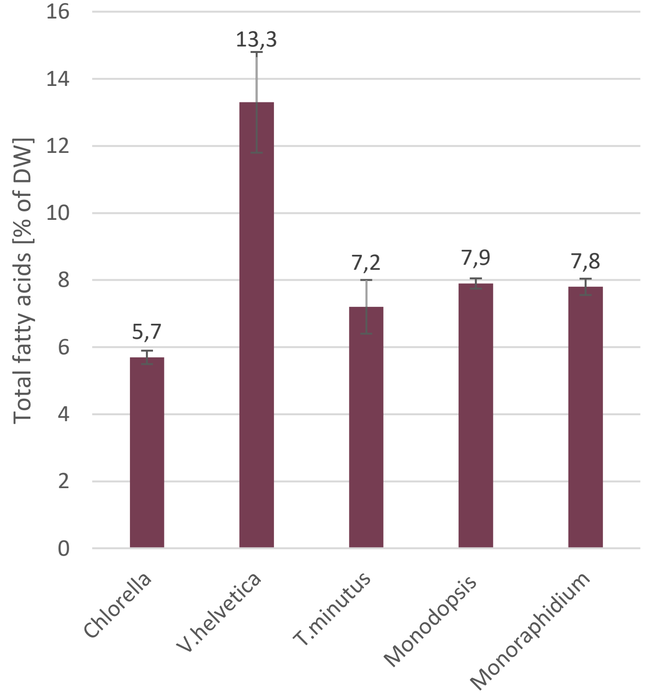


Fig. 27 Total FA content of selected microalgae grown in AC-PBR.

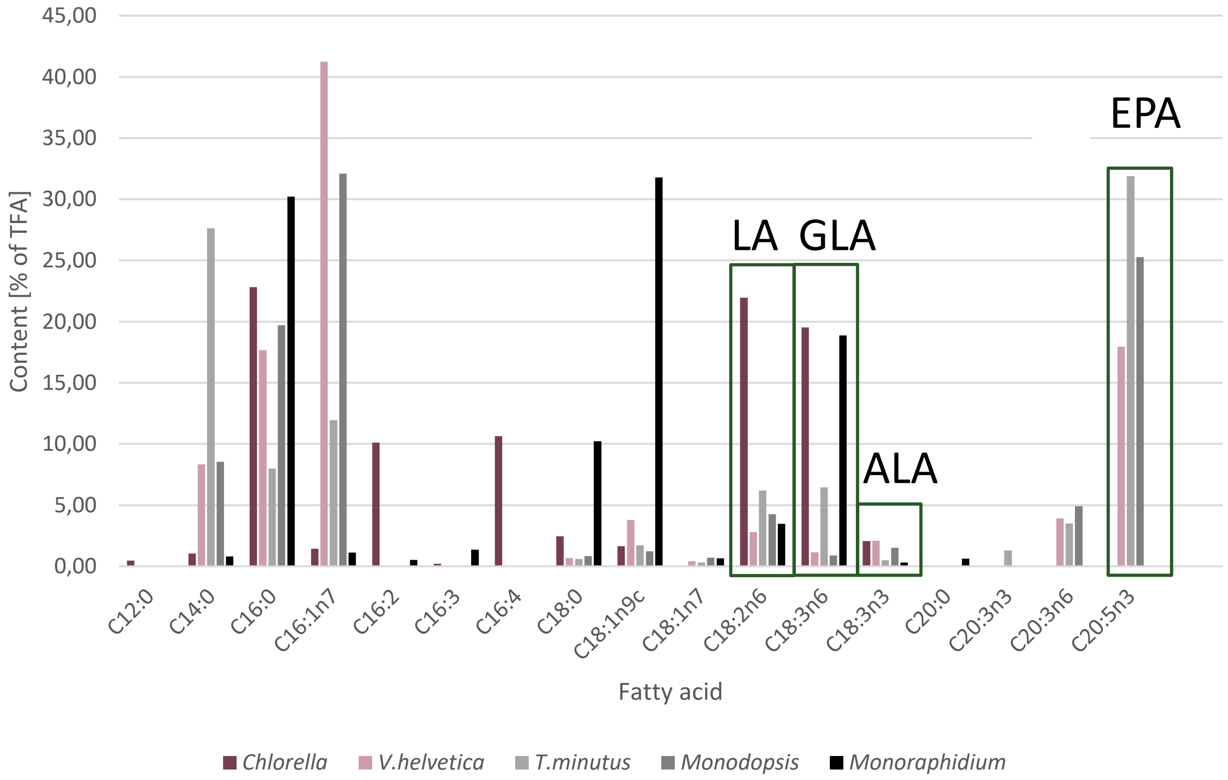
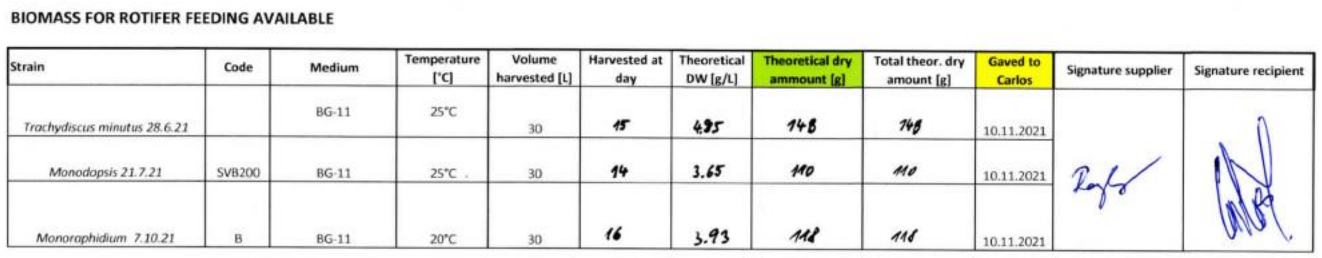


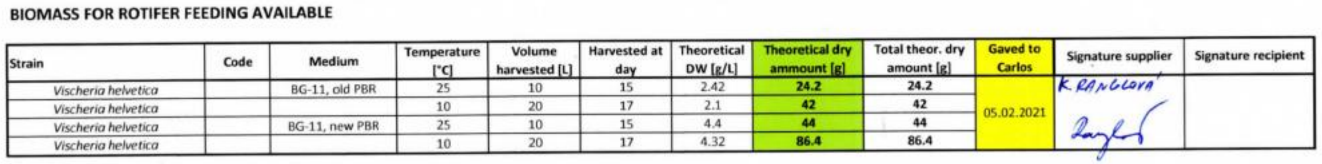
Fig. 28 Individual FA content of selected microalgae grown in AC-PBR.

BIOMASS HANDOVER



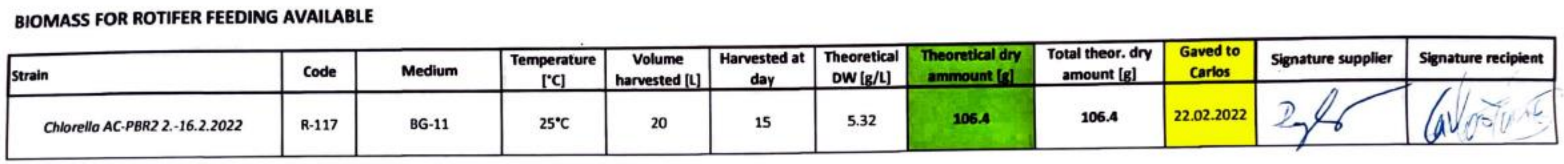
BIOMASS FOR ROTIFER FEEDING AVAILABLE

Strain	Code	Medium	Temperature [°C]	Volume harvested [L]	Harvested at day	Theoretical DW [g/L]	Theoretical dry ammount [g]	Total theor. dry amount [g]	Gaved to Carlos	Signature supplier	Signature recipient
Trachydiscus minutus 28.6.21		BG-11	25°C	30	15	4.95	148	148	10.11.2021		
Monodopsis 21.7.21	SVB200	BG-11	25°C	30	14	3.65	110	110	10.11.2021		
Monoraphidium 7.10.21	B	BG-11	20°C	30	16	3.93	118	118	10.11.2021		



BIOMASS FOR ROTIFER FEEDING AVAILABLE

Strain	Code	Medium	Temperature [°C]	Volume harvested [L]	Harvested at day	Theoretical DW [g/L]	Theoretical dry ammount [g]	Total theor. dry amount [g]	Gaved to Carlos	Signature supplier	Signature recipient
Vischeria helvetica		BG-11, old PBR	25	10	15	2.42	**24.2**	**24.2**	05.02.2021	K. RANGLOVÁ	
Vischeria helvetica			10	20	17	2.1	**42**	**42**			
Vischeria helvetica		BG-11, new PBR	25	10	15	4.4	**44**	**44**			
Vischeria helvetica			10	20	17	4.32	**86.4**	**86.4**			



BIOMASS FOR ROTIFER FEEDING AVAILABLE

Strain	Code	Medium	Temperature [°C]	Volume harvested [L]	Harvested at day	Theoretical DW [g/L]	Theoretical dry ammount [g]	Total theor. dry amount [g]	Gaved to Carlos	Signature supplier	Signature recipient
Chlorella AC-PBR2 2.-16.2.2022	R-117	BG-11	25°C	20	15	5.32	**106.4**	**106.4**	22.02.2022		

OUTPUTS – PUBLICATIONS (3 IN TOTAL)

applied sciences

Review

Digestate as Sustainable Nutrient Source for Microalgae—Challenges and Prospects

Lisa Bauer [1], **Karolína Ranglová** [2,3], **Jiří Masojídek** [2,4], **Bernhard Drosg** [1,5] **and Katharina Meixner** [1,5,*]

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[3] Faculty of Agriculture, University of South Bohemia, 370 05 České Budějovice, Czech Republic
[4] Faculty of Science, University of South Bohemia, 370 05 České Budějovice, Czech Republic
[5] Institute for Environmental Biotechnology, Department IFA-Tulln, University of Natural Resources and Life Sciences, Vienna, Konrad Lorenz Straße 20, 3430 Tulln, Austria
* Correspondence: katharina.meixner@best-research.eu

Featured Application: This review provides insight into the origin of digestate and how it can be processed to meet requirements for microalgae cultivation as well as challenges that occur in cultivation, in downstream processing, and in terms of products when using this nutrient source.

Abstract: The interest in microalgae products has been increasing, and therefore the cultivation industry is growing steadily. To reduce the environmental impact and production costs arising from nutrients, research needs to find alternatives to the currently used artificial nutrients. Microalgae cultivation in anaerobic effluents (more specifically, digestate) represents a promising strategy for increasing sustainability and obtaining valuable products. However, digestate must be processed prior to its use as nutrient source. Depending on its composition, different methods are suitable for removing solids (e.g., centrifugation) and adjusting nutrient concentrations and ratios (e.g., dilution, ammonia stripping). Moreover, the resulting cultivation medium must be light-permeable. Various studies show that growth rates comparable to those in artificial media can be achieved when proper digestate treatment is used. The necessary steps for obtaining a suitable cultivation medium also depend on the microalgae species to be cultivated. Concerning the application of the biomass, legal aspects and impurities originating from digestate must be considered. Furthermore, microalgae species and their application fields are essential criteria when selecting downstream processing methods (harvest, disintegration, dehydration, product purification). Microalgae grown on digestate can be used to produce various products (e.g., bioenergy, animal feed, bioplastics, and biofertilizers). This review gives insight into the origin and composition of digestate, processing options to meet requirements for microalgae cultivation and challenges regarding downstream processing and products.

check for updates

Citation: Bauer, L.; Ranglová, K.; Masojídek, J.; Drosg, B.; Meixner, K. Digestate as Sustainable Nutrient Source for Microalgae—Challenges and Prospects. *Appl. Sci.* **2021**, *11*, 1056. https://doi.org/10.3390/app11031056

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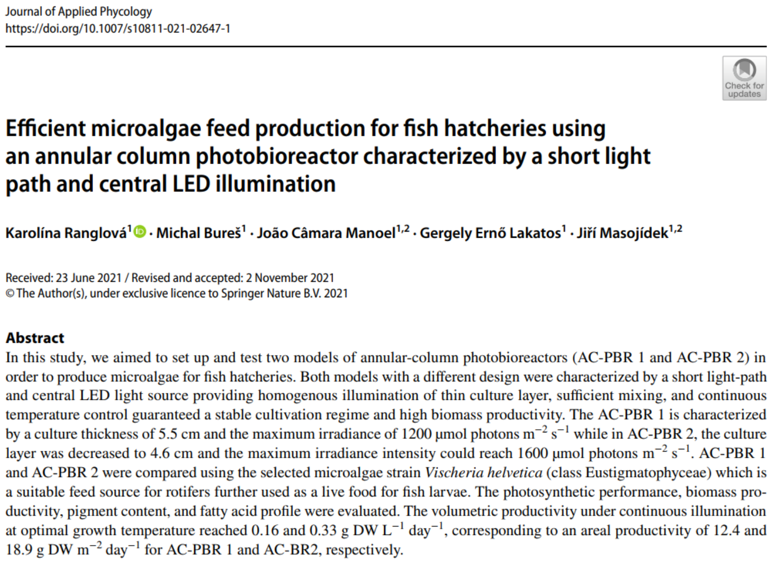

Journal of Applied Phycology
https://doi.org/10.1007/s10811-021-02647-1

Check for updates

Efficient microalgae feed production for fish hatcheries using an annular column photobioreactor characterized by a short light path and central LED illumination

Karolína Ranglová[1] · **Michal Bureš**[1] · **João Câmara Manoel**[1,2] · **Gergely Ernő Lakatos**[1] · **Jiří Masojídek**[1,2]

Received: 23 June 2021 / Revised and accepted: 2 November 2021

Abstract
In this study, we aimed to set up and test two models of annular-column photobioreactors (AC-PBR 1 and AC-PBR 2) in order to produce microalgae for fish hatcheries. Both models with a different design were characterized by a short light-path and central LED light source providing homogenous illumination of thin culture layer, sufficient mixing, and continuous temperature control guaranteed a stable cultivation regime and high biomass productivity. The AC-PBR 1 is characterized by a culture thickness of 5.5 cm and the maximum irradiance of 1200 µmol photons m^{-2} s^{-1} while in AC-PBR 2, the culture layer was decreased to 4.6 cm and the maximum irradiance intensity could reach 1600 µmol photons m^{-2} s^{-1}. AC-PBR 1 and AC-PBR 2 were compared using the selected microalgae strain *Vischeria helvetica* (class Eustigmatophyceae) which is a suitable feed source for rotifers further used as a live food for fish larvae. The photosynthetic performance, biomass productivity, pigment content, and fatty acid profile were evaluated. The volumetric productivity under continuous illumination at optimal growth temperature reached 0.16 and 0.33 g DW L^{-1} day^{-1}, corresponding to an areal productivity of 12.4 and 18.9 g DW m^{-2} day^{-1} for AC-PBR 1 and AC-BR2, respectively.

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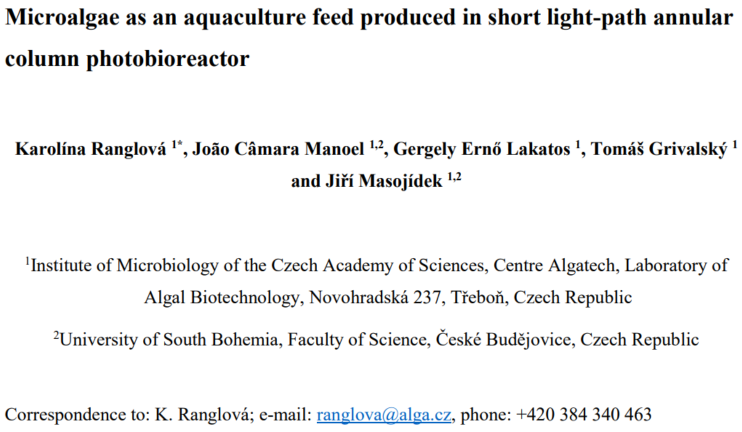

Microalgae as an aquaculture feed produced in short light-path annular column photobioreactor

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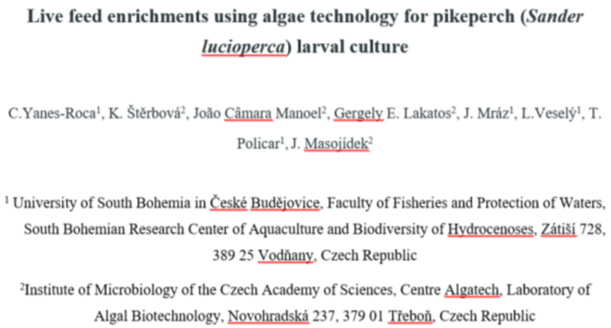

Live feed enrichments using algae technology for pikeperch (*Sander lucioperca*) larval culture

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In progress

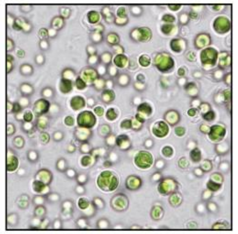
OUTPUTS – OTHERS

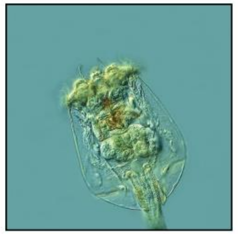
- Science fairs
- Science nights
- Summer school Ypsertal
- Regular seminars at Algatech
- Poster Session Algatech
- ... others

SUMMARY

- **5 microalgae strains were selected** on the base of the growth and FA profile
- All of them **characterized** in small scale an AC-PBR as well
- **3 AC-PBRs were completed** and 2 of them were delivered to our partners
- All **microalgae provided to BEST and FF Vodňany** for further test

THANK YOU FOR YOU ATTENTION





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